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SERRATIA MARCESCENS BIZIO AS AN INSECT PATHOGEN[1]

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INTRODUCTION

Serratia marcescens Bizio is a small, gram-negative, rodshaped bacterium characterized by the production of a red pigment. Generally speaking, it is a saprophytic organism commonly found in water and soil, and in milk, bread, and other foods. It has been known by several synonyms, including *Bacillus prodigiosus* (Flügge), *Bacterium prodigiosum* (Lehm. and Neum.), and *Chromobacterium prodigiosum* (Top. and Wil.). Under ordinary circumstances it is nonpathogenic to vertebrates except in enormous doses. It has been found associated with insects in several ways and under a variety of conditions (Steinhaus, 1946, 1949). In the present paper we are primarily concerned with it as a pathogen for insects.

Of the three species of *Serratia* (tribe Serrateae, family Enterobacteriaceae) that have been recorded as being associated with insects, only *Serratia marcescens* has been known, at times, to cause disease in insects. Cao (1906*a*, *b*) used *Serratia kilensis* (Lehm. and Neum.), as well as *S. marcescens*, to demonstrate the ability of flies to transmit bacteria. Inasmuch as the experimental flies died somewhat sooner than usual, it is possible that the bacteria played some role in their deaths. *S. kilensis* was originally isolated from water and is presumed to be widely distributed. A third species, *Serratia plymuthicum* (Lehm. and Neum.), has been isolated (Steinhaus, 1941) from numerous specimens of the cricket *Neombius fasciatus* var. *fasciatus* DeGeer collected in nature in Ohio. Since the crickets appeared to be healthy and normal in every respect, the bacterium was considered to be a saprophytic inhabitant of their alimentary tract. Ordinarily it is found in water and various foods.

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Previous Reports

The great majority of reported instances in which *S. marcescens* has been responsible for causing disease in insects have been concerned with laboratory- or insectary-reared insects. In spite of the rather ubiquitous distribution of the bacterium, there have been only a few reports of *S. marcescens*-infected insects having been found in nature.

Beginning with Rozier, in 1796 and 1817, early sericulturists occasionally observed that the bodies of dead silkworms, *Bombyx mori* (Linn.), assumed a red coloration which presumably, in some cases, might have indicated the presence of the red-pigmented bacterium. In most instances—there are at least 20 such nineteenth-century reports—the reddish color was described in larvae infected with the fungus, *Beauveria bassiana* (Bals.), of white muscardine. Although it is now known that this fungus itself may, at times, impart a pinkish cast to insects it infects, some workers (e.g., Perroncito, 1886) have attempted to show that the color is caused by a simultaneous infection with *S. marcescens*. That such is occasionally the case has been confirmed (see Masera, 1936*c*).

Serratia marcescens has been found infecting the silkworm by a number of workers (e.g., Tateiwa, as referred to by Metalnikov and Chorine, 1928, 1929 *a, b;* Nomura, 1902; Carbone and Fortuna, 1932). Experimental inoculations of *S. marcescens* into the body cavity of the silkworm were also accomplished by early workers such as Aoki and Chigasaki (1915) who found the bacterium to be highly lethal even in minute doses. They also observed that in the hemocoele the bacterium underwent rather remarkable morphological changes, becoming extraordinarily long and thick. On the other hand, Sawamua (1905) obtained no mortality in silkworms either by feeding the bacterium or by inoculating it directly into the hemocoele. Masera (1934 *a,b,c,d;* 1936 *a,b,c;* 1937) and others have found that experimentally the silkworm is susceptible to *S. marcescens* by injection, but only rarely by ingestion. When ingested, the bacterium is pathogenic in varying degrees depending on the stage of the larva; the mortality is greater in the later than in the earlier instars. Masera found that when the bacterium is fed to silkworms it may frequently be reisolated from the larvae and from the subsequent pupae, but not from the adults or eggs. However, back in 1885 Bandelli found the bacterium in naturally dead moths in his laboratory; and Broquet (1910) reported a similar finding from moths in Indo-China. Masera also observed an interesting antagonistic effect between *S. marcescens* and *Beauveria bassiana* both *in vitro* and in larvae of the yellow mealworm, *Tenebrio molitor* Linn.; and Dresner (1950), as well as Masera, has reported the *in vitro* inhibition of *S. marcescens* growth by *B. bassiana*. The bacterium is not considered to be pathogenic for the larva of the mealworm, but the fungus is. However, when the insect is exposed to both organisms simultaneously, the activity of the fungus is inhibited, which results in a lower larval mortality. Because the development of the fungus in culture is inhibited by *S. marcescens*, Masera assumes that the same process takes place in the insect.

Some interesting experiments by Masera (1954) concerning the bacteri-

cidal properties of the digestive juices of silkworms involve the use of *S. marcescens* as a test organism. According to this author, the intestinal juice of the silkworm tends to be bactericidal for the bacterium both *in vivo* and *in vitro*. This fact may explain Masera's observations that *S. marcescens*, after being ingested by a silkworm, is usually destroyed, or at least greatly diminished in numbers. *In vitro*, at 25°C, the intestinal juices kill the bacterium within 24 hours. The active principle is thermostable at temperatures as high as 100°C. It maintains its bactericidal power, with slight diminution, for at least 17 months. It loses its potency when diluted out at a ratio of 1:50. In addition to its bactericidal properties the active principle appears to be virucidal (silkworm polyhedrosis virus) and fungicidal (*Beauveria*); the latter property, however, appears to be affected by heat (55° and 100°C). There appears to be a quantitative relation between the amount or potency of the bactericidal element and the number of bacteria. By increasing the number of bacterial cells in a given amount of intestinal juice, a point is reached at which the bactericidal properties of the juice are no longer in evidence. Masera calls this phenomenon "stanchezza" (tiredness, exhaustion). As the point of "stanchezza" is approached, Masera found that rose-colored variants or mutations arose upon subculturing the red-pigmented *S. marcescens* originally placed in the juice. This saturation effect may also be demonstrated in the living insects by feeding them leaves contaminated with the bacterium. Masera infers that, as far as its effect upon *S. marcescens* is concerned, a similar active principle is in other insects (e.g., *Arge*, *Pieris*). Whether there is any relation between this substance and that reported in other insects by other authors (e.g., Duncan, 1926) is not made clear.

In addition to the silkworm, a considerable number of other insects have been reported to be susceptible to both natural and experimental infections caused by *S. marcescens*. In 1915, Barber and Jones found that locusts in the Philippines died following the direct inoculation of the bacterium. However, when the bacteria were sprayed on the food of the locusts both in the laboratory and in the field only a small amount of mortality resulted. In the course of experiments designed to study the principles of infection and immunity in insects, Metalnikov (1920, 1930 *a*, *b*) and Zernoff (1931) found the larva of the wax moth, *Galleria mellonella* (Linn.), to be highly susceptible to the bacterium when the latter was injected into the hemocoele, but quite resistant to infection *per os*. These authors explained the difference in susceptibility by the two routes on the assumption that *Galleria*, because it lives on food open to contamination, possesses a naturally acquired immunity which enables them to resist bacterial infection through the digestive tract. Masera holds a similar view with regard to the resistance of *Tenebrio:* that through the normal feeding on contaminated food the insect develops an immunity to *S. marcescens*. It would appear that this generalization is in need of experimental confirmation. One of the strains of *S. marcescens* used by Metalnikov had been isolated from a diseased gypsy-moth larva, *Porthetria dispar* (Linn.), for which the bacterium was markedly pathogenic when tested, by feeding, in the laboratory. About this same time Metalnikov and Chorine (1928, 1929*a*,*b*) and Zernoff (1931) found the

European corn borer, *Pyrausta nubilalis* (Hbn.), to be susceptible to the bacterium by intrahemocoelic injection, and at least moderately so by feeding. And, in 1934, Masera reported that the yellow mealworm, *Tenebrio molitor* (Linn.), was not susceptible to *S. marcescens* when administered by any route. (As we shall have occasion to point out later, however, our own experiments with the mealworm have given somewhat different results.) Thus, in the examples so far cited we can see what appears to be a gradation of susceptibility from that of the corn borer, which is susceptible to *S. marcescens* by direct inoculation as well as through the digestive tract, to the silkworm which is susceptible by inoculation but irregularly so by ingestion, to the larva of the wax moth which is susceptible to injection but not by ingestion, to the yellow mealworm which, according to some authors, is not susceptible by either route. As we shall explain later, however, these degrees of susceptibilty are not invariable and there are exceptions to them.

In 1934, Martchuk conducted a series of experiments in which he found that in the laboratory larvae of *Loxostege sticticalis* (Linn.), *Pieris brassicae* (Linn.), *Malacosoma neustrium* (Linn.), and *Hyponomeuta malinella* Zeller were rather highly susceptible to *S. marcescens*. In recognition of these results, Kolesnik (1938) reports that *S. marcescens* has no pathogenic effect on the honey bee at any stage of the insect's development. He felt that this finding was important in that it made it possible to attempt the use of the bacterium as a microbial control agent without endangering apiculture.

The possibility of controlling insects by using *S. marcescens* has been investigated by Drobotjko, Martchuk, Bisenman, and Sirotskaya (1938). They found that, in the laboratory, when the bacterium was administered (along with food) to the larvae of *Loxostege sticticalis* mortality of 60–69 per cent resulted. However, in the field the mortality was much lower or very slight. The authors believe that the results of their field experiments were influenced by meteorological conditions as well as by the fact that the bacterium had lost its virulence on being held under laboratory conditions. In 1940 Beltiukova and Romanevich reported the susceptibility of the beet weevil *Cleonus punctiventris* Germ. to *S. marcescens* in laboratory and field tests. They also report that occasionally they had found larvae naturally infected with the bacterium.

In the course of rearing colonies of the desert locust, *Schistocerca gregaria* Forsk., Lepesme (1937*a*,*b*) observed an outbreak of disease caused by *S. marcescens*. Experimentally he found that the bacterium freshly isolated from dead insects killed the locusts within 12 hours when it was inoculated directly into the hemocoele, and within 24 hours when administered perorally. After the organism had been grown for several transfers on nutrient agar, it lost its virulence to a degree that fatal infections (after 3 days) could be induced only by direct inoculation. Among other observations, Lepesme noticed that in diseased locusts *S. marcescens* appeared to be much more abundant in the blood of the insect than in its digestive tract; that later stages of *Schistocerca* are more susceptible than early stages; and that the bacterium is probably capable of infecting the embryo of the insect as evidenced by internal red coloration once seen in eggs from an infected female.

Another instance of the appearance of disease caused by *S. marcescens* in laboratory-reared insects has been reported by DeBach and McOmie (1939). The disease broke out from time to time and with varying degrees of severity in stocks of the termite *Zootermopsis angusticollis* Hagen being reared for studies of caste differentiation. They found that a 50 per cent mortality resulted when the bacterium was fed to the termites, and a 90 to 100 per cent mortality when it was inoculated into the body cavity.

Swain (1945) found larvae of white-fringed beetles (*Pantomorus* spp.) to be susceptible to *S. marcescens* when the bacterium was inoculated into the body cavity.

S. marcescens has been observed to cause infection and considerable mortality in the potato tuberworm, *Gnorimoschema operculella* (Zeller), being mass-reared in an insectary (Steinhaus, 1945). The susceptibility of the insect to the bacterium was confirmed by peroral and direct inoculation infectivity tests. Infected larvae become sluggish in movement, less sensitive to external stimuli, have a diminished appetite, and frequently are diarrheic. Upon death, the insects become bright red in color, but turn brown upon subsequent disintegration. Insect parasites, such as *Macrocentrus ancylivorus* Roh. and *Dibrachys cavus* Wlk., reared on tuberworms frequently acquire the infection from the host insects.

While investigating the passage of microorganisms through the digestive tract of the roach *Blaberus cranifer* Burm., Wedberg, Brandt, and Helmboldt (1949) observed that extensive multiplication of *S. marcescens* occurred in some roaches fed with the bacterium. These insects frequently died shortly after a deep-red color appeared in the "upper half" of their bodies. It was assumed that the bacteria were at least a contributing factor in the death of the roaches. An occasional death of a test arthropod (e.g., ticks and midges) has been observed in other instances in which *S. marcescens* was used as an indicator organism (e.g., see Steinhaus, 1942, and Steinhaus and Brinley, 1957).

The susceptibility of coleopterous stored-grain insects to *S. marcescens* has been found to be slight when administered along with the insects' food. Only an occasional specimen of the following species has been observed (Steinhaus and Bell, 1953) to die from infection by the bacterium: the granary weevil, *Sitophilus granarius* (Linn.); the rice weevil, *Sitophilus oryza* (Linn.); and the confused flour beetle, *Tribolium confusum* (Duv.). The angoumois grain moth, *Sitotroga cerealella* (Oliv.), was found to be resistant to 3 strains of *S. marcescens* fed to the larvae.

Heimpel (1955*a*), using a strain of *S. marcescens* previously isolated by June Stephens from larvae of the codling moth, *Carpocapsa pomonella* (Linn.), tested (by feeding in the laboratory) the pathogenicity of the bacterium for 5 species of sawflies. He obtained mortalities of 63 per cent for *Pristiphora erichsonii* (Htg.) and *Neodiprion lecontei* (Fitch), 59 per cent for *Neodiprion banksianae* Roh., 47 per cent for *Nematus ribesii* (Scop.), and 44 per cent for *Neodiprion swainei* Midd.

Serratia, presumably *S. marcescens*, has been found associated with wasps from nests showing signs of abortive brood. Strains of the bacterium have been isolated from the intestines of larvae of *Polistes gallicus* Linn., *Polistes*

nympha, and from abandoned nests of *P. gallicus* and *Vespula germanica* (Fabr.) (Vergé, 1952).

Vago and Vasiljević (1954) refer to a strain of *S. marcescens* isolated from "*Epicampoter* sp." Vasiljević (1957) found that butterflies of the fall webworm, *Hyphantria cunea* (Drury), that had emerged from infected pupae frequently turned red after a time. However, these butterflies showed no symptoms of disease.

Among diseased insects received for diagnoses, Steinhaus (1951) reports *S. marcescens* as the pathogen in pupae of the mourning cloak butterfly, *Nymphalis antiopa* (Linn.); adult houseflies, *Musca domestica* Linn.; the termite, *Zootermopsis angusticollis* (Hagen); and insectary-reared oriental fruit flies, *Dacus dorsalis* Hendel. As indicated in Table 1, other diagnoses since then have included isolations from the American cockroach, *Periplaneta americana* (Linn.), the sweetpotato weevil, *Cylas formicarius elegantulus* (Sum.), the yellow mealworm, *Tenebrio molitor* Linn., the rhinoceros beetle, *Oryctes rhinoceros* Linn., the corn earworm, *Heliothis zea* (Boddie), and the mountain pine beetle, *Dendroctonus monticolae* Hopk.

The literature contains a number of references to red-pigmented bacteria associated with insects, and which may represent strains of *S. marcescens*. We have already referred to eighteenth and nineteeth century accounts of pink to red-colored silkworms which, in some cases, may have been infected with *S. marcescens*. In 1909 Reiff referred to "reddish pupae" of *Junonia coenia* Hübner. Strickland (1916) reports the occurrence of a bacterial disease in larvae of the army cutworm, *Chorizagrotis auxiliaris* (Grote), which turned "an opaque pink" color. The disease occurred among insects both in nature and held in cages. (In the same report Strickland mentions another disease of the cutworm which might have been either one of the virus diseases known in this insect [Steinhaus, 1957].) In 1932, Zernoff referred to a "Bacille d'Oceneria rouge" as being experimentally pathogenic for *Carausius morosus* Brunner. Possibly this represents a strain of *S. marcescens* isolated from a species of *Oceneria*. Recently, Lysenko (1958) has determined his strain of *Bacillus noctuarum* White *(Bacterium noctuarum, Pseudomonas noctuarum)* to be *Serratia marcescens*.

The Problem

From the foregoing brief review of the literature it is apparent that as yet the reputed role of *Serratia marcescens* as a pathogen of insects is based largely on the occurrence of outbreaks among laboratory or insectary-reared insects and on experimental infections rather than on any marked degree of spontaneous infection among insects in nature. In 1945, the author circulated an inquiry to, and received replies from, approximately 100 American entomologists as to whether or not they had ever observed or known of the occurrence of disease in insects caused by red-pigmented bacteria. Ten of the entomologists responding to the questionnaire mentioned having observed pink coloration in insects they had found dead or dying, usually in the laboratory. However, none of the replies testified as to the proved presence of *S. marcescens* in the diseased insects.

During the thirteen years our Laboratory has been receiving specimens of

diseased insects for diagnosis, we have never, with one exception, received any specimens, representing a true epizootic in nature, that were infected with *S. marcescens*. In most instances, the *Serratia*-infected specimens received represented insectary rearings or had been taken from laboratory rearing cages. The one possible exception was a single dead larva of *Heliothis zea* (Boddie) sent to us by J. H. Lilly who collected it from a field of corn in southwestern Iowa.[3] Cultures from this specimen yielded a strain of *S. marcescens*. Thus, although *S. marcescens* occurs rather ubiquitously in nature, it does not, in general, appear to be responsible for widespread epizootics among insects in nature, and emerges as a pathogen primarily among insects being reared in laboratories or insectaries. The same situation undoubtedly also pertains to certain other bacteria occasionally found causing disease in laboratory-held specimens but not in the field.

This state of affairs is probably open to a number of explanations, most of them of an epizootiological nature. However, one facet of this problem has been of interest to us in the course of our work on the relation of stress to disease in insects. It concerns the questions: To what extent is the occurrence of *Serratia* infections in the laboratory (as distinguished from the relative rarity of such infections in nature) related to factors of stress that may be present under certain laboratory conditions but do not necessarily prevail in nature? Can these same stressors be applied in the field so as to make the bacterium a more effective pathogen, and possibly a potential control agent? While the results of our investigation to date do not provide definitive answers to these questions, they do give some indication of the nature of the problems involved. The primary purpose of the present paper, in addition to reviewing the literature briefly, is to bring together the results of miscellaneous studies we have conducted over the past several years, and to "clear the way" for possible future reports on research of a more definitive nature involving this bacterium.

MATERIALS AND METHODS

A total of 33 strains, or isolates, of *S. marcescens* was used at different times in the experiments recorded in the present paper. Following standard procedures of determinative bacteriology, all strains proved to be typical of *Serratia marcescens* Bizio. Furthermore, all strains produced moderate to bright-red pigment and showed little tendency to dissociate into white and pink variants. Most of the strains had been originally isolated from insects, but some were from other sources (see Table 1). Strains 0–8–2 through 0–8–6 were obtained in 1949 from the late Professor R. S. Breed who had received them in 1927 from Professor E. Hiratsuka, then Director of the Imperial Japanese Sericultural Experiment Station near Tokyo, Japan. They had been isolated from diseased silkworms at that institution. Strain 0–8–9 was originally isolated by Dr. H. Kufferath from a dead locust in Greece. Before

[3] After this was written, we received from J. J. H. Szent-Ivany of Port Moresby, in the Territory of Papua and New Guinea, dead specimens of a noctuid, *Pericyma cruegeri* Butler. These larvae were collected in nature from beneath a *Poinciana* tree near his laboratory. From some of the insects we isolated a strain of *Serratia marcescens* (79–1–1). The role of this bacterium in the natural mortality of the insect has not been determined.

sending it to Professor Breed, he had found it pathogenic for some unidentified caterpillars when fed to the insects.

For the most part, the insects used in this investigation were reared in our laboratory. The species concerned were: the alfalfa caterpillar, *Colias philodice eurytheme* Boisduval [Pieridae], the buckeye caterpillar, *Junonia coenia* Hübner [Nymphalidae], the silkworm, *Bombyx mori* (Linnaeus) [Bombycidae], the salt-marsh caterpillar, *Estigmene acrea* (Drury) [Arctiidae], the variegated cutworm, *Peridroma margaritosa* (Haworth) [Phalaenidae], the armyworm, *Pseudaletia unipuncta* (Haworth) [Phalaenidae], the omnivorous looper, *Sabulodes caberata* Guenèe [Geometridae], the greater wax moth, *Galleria mellonella* (Linnaeus) [Galleriidae], and the yellow mealworm, *Tenebrio molitor* Linnaeus [Tenebrionidae].

The techniques used in administering the bacteria to the insects conform with those reported in other similar studies made in this Laboratory. Intrahemoceolic inoculations were made using sharply-pointed dissecting needles or the Dutky-Fest microinjector. *S. marcescens* was fed to the test insects by giving them food dipped in aqueous suspensions of the bacteria, or, more often, by using the Martignoni microinjector needle (Martignoni, 1955) adapted to a Dutky-Fest microinjector. In the present paper we have designated the latter method of administration as "microfeeding," when applied *per os,* and "microinjection" when introduced through the integument directly into the body cavity. In the case of the former, amounts of 0.003 and 0.006 ml were used, while in the case of the latter 0.003 ml was used. A roughly uniform concentration of bacteria was arrived at by suspending two large loopsful of culture growth from a 24- to 48-hour nutrient agar slant in 2 ml of sterile distilled water just prior to use. Based on plate counts, it was calculated that an amount of 0.003 ml of this suspension contained approximately 325,000 viable bacterial cells.

In the different experiments precautions were taken, insofar as was possible, to eliminate or account for the effects of all known stressors, such as crowding (Steinhaus, 1958*a*), other than the one being tested.

EXPERIMENTATION AND RESULTS

General Susceptibility Tests

On the basis of susceptibility tests using the nine species of insects already indicated, we have been able to confirm the generally held concept that while *Serratia marcescens* is highly pathogenic for many insects upon direct inoculation into the body cavity, it is considerably less so when fed to them. It might be pointed out that the pathogenesis of *S. marcescens* infections in insects has never been worked out in detail. In general, it may be said that following ingestion of the bacterium, and after a variable incubation period, usually 20 to 48 hours, the insect dies of a typical septicemia. The bacterium frequently imparts a pink or red color to the insect's body just before or for a short while after death. Ordinarily, as post-mortem changes occur, the color of the insect gradually darkens and becomes a dark brown or black. The blood and tissues of the dying insect are filled with the bacterium which is easily isolated and readily cultivable. (Figs. 1–6 and Plate I.)

In one experiment in which larvae were fed food dipped in aqueous suspensions of the bacterium (strains 0–8–2 through 55–1–1, as indicated in Table 1), it was found that 70.4 per cent of *Sabulodes*, 65.8 per cent of *Junonia*, and 7.8 per cent of *Pseudaletia* died of septicemia caused by *S. marcescens*. Six larvae were used in testing each strain. Combining the results of tests of all three insects, it was noted that the mortality according to the

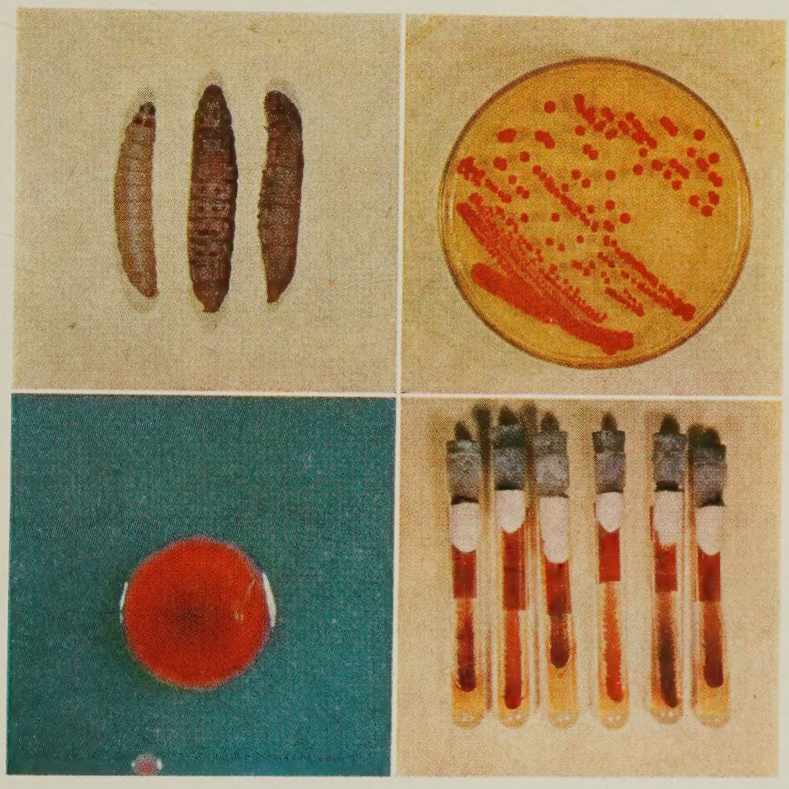

Plate I. *Upper left:* Larvae of the greater wax moth, *Galleria mellonella* (Linnaeus). Individual on the left is healthy. The remaining two are dead, having been microinjected with *Serratia* (0–8–4) 28 hours previously. *Upper right:* Colonies of *Serratia marcescens* Bizio after growing 48 hours on nutrient agar. Incubated at 28°C. *Lower left:* Close-up of single colony of *S. marcescens*. *Lower right:* Six different, but typical, strains of *S. marcescens* grown on nutrient agar at 28°C for 48 hours, and held in the refrigerator (5°C) for one month. These cultures show the deep red color typical of most of the entomogenous strains of the bacterium used in this investigation.

different strains varied from a low of 8.3 per cent for strain 0–8–9 to a high of 66.7 per cent for strain 0–8–12.

During the course of our experiments, a number of susceptibility tests of different types were run. The results obtained may be summarized as follows:

Using 24- to 48-hour cultures of strain 0–8–13, larvae of *Galleria* showed a mortality of about 5 per cent when the bacterium was incorporated in the insect's food (pablum-honey-glycerin mixture), about 20 per cent when

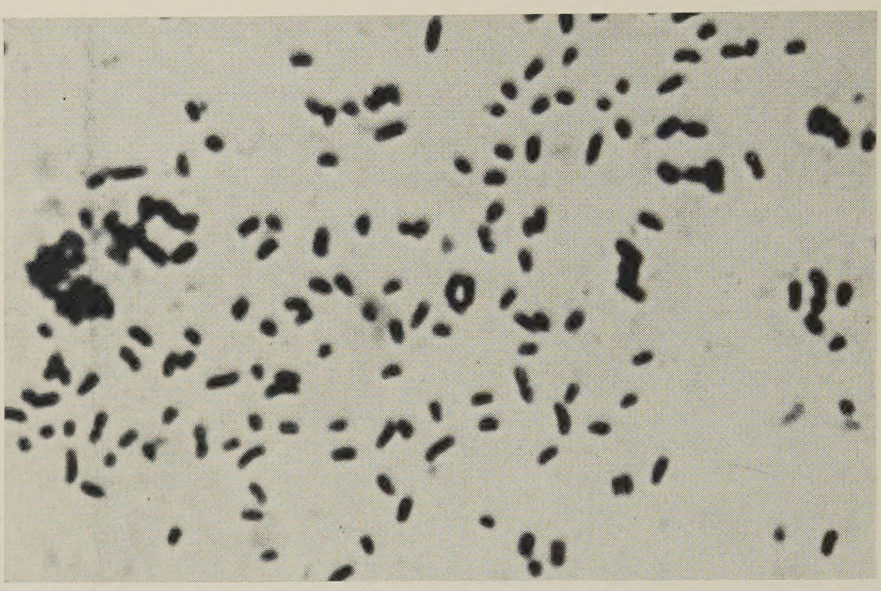

Fig. 1. Photomicrograph of *Serratia marcescens* Bizio (strain 0–8–4) in the blood of a dead *Galleria* larva, 24 hours after having been inoculated with the bacterium. Stained with crystal violet.

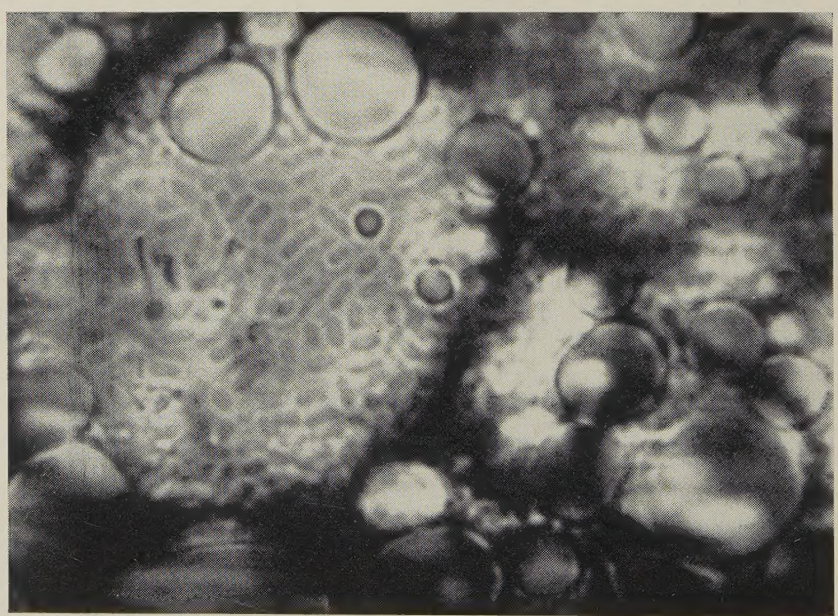

Fig. 2. Photomicrograph of a wet mount of the fat tissue from a *Galleria* larva dead of infection with *Serratia* (0–8–4) that had been inoculated 24 hours earlier.

Fig. 3. Larvae of the silkworm, *Bombyx mori* (Linnaeus), showing changes in the external appearance of caterpillars dead of *Serratia* infection (24 hours after inoculation with strain 0–8–4) [the two lower individuals], as compared with a healthy insect [the top individual].

Fig. 4. The alfalfa caterpillar, *Colias philodice eurytheme* Boisduval, showing different manifestations of change (darkening of integument) in the external appearance at the time of death, 24 hours following the inoculation of *Serratia* (0–8–4). Larva at the top is a healthy individual.

Fig. 5. The variegated cutworm, *Peridroma margaritosa* (Haworth), showing changes (mostly a generalized darkening of the integument) in the external appearance shortly after death from *Serratia* (0–8–4) infection. Infected larva at top; healthy larva at bottom.

Fig. 6. The yellow mealworm, *Tenebrio molitor* Linnaeus, showing (upper larva) changes (largely postmortem) resulting from infection, by puncture, with *Serratia* (0–8–4), as compared with healthy insect (lower larva). Photograph taken 48 hours after death.

microfed (0.006 ml of a bacterial suspension: 5 ml of water per agar slant), and about 85 per cent when the integument was punctured with a bacteria-laden needle. Smaller larvae appeared to be slightly less susceptible than larger, i.e., older, larvae. Thirty-five larvae were used in each test. In the tests in which the bacterium was incorporated in the food, it did not appear to

Table 1. Strains, or isolates, of *S. marcescens* used in experiments, and their sources.

Our strain no.	Insect, or other source, from which originally isolated	Person or laboratory from which obtained	Original strain or accession no.
0–8–2	*Bombyx mori* Linn.	R. S. Breed (from E. Hiratsuka)	J21
0–8–3	*Bombyx mori* Linn.	R. S. Breed (from E. Hiratsuka)	JB160
0–8–4	*Bombyx mori* Linn.	R. S. Breed (from E. Hiratsuka)	JWSA
0–8–5	*Bombyx mori* Linn.	R. S. Breed (from E. Hiratsuka)	JRS
0–8–6	*Bombyx mori* Linn.	R. S. Breed (from E. Hiratsuka)	JB161
0–8–7	Not indicated	R. S. Breed	18–1–3
0–8–8	Not indicated	R. S. Breed	1ZH
0–8–9	"Locust from Greece"	R. S. Breed (from H. Kufferath)	7
0–8–10	Not indicated	R. S. Breed	2–23–1
0–8–11	Not indicated	R. S. Breed	6–47
0–8–12	Not indicated	R. S. Breed	6–33
0–8–13	Not indicated	R. S. Breed	6–42
0–9–1	Apples	J. M. Stephens	SM (JS)
0–9–2	Used in housefly and Drosophila studies	J. M. Stephens	SM (OK)
0–9–3	Laboratory stock—source unknown	J. M. Stephens	SM (K)
0–9–4	Unknown. Possibly of human origin	J. M. Stephens	SM (H)
0–10–1	*Malacosoma*	J. M. Stephens	MA2502
0–10–2	*Malacosoma*	J. M. Stephens	MA2532
0–11–1	*Dacus dorsalis* Hendel	S. Maeda	
0–11–2	*Dacus dorsalis* Hendel	S. Maeda	
0–11–3	*Dacus dorsalis* Hendel	S. Maeda	
28–2–1	*Musca domestica* Linn.	Lab. of Insect Path., U.C.	Acc.394
53–1–1	*Periplaneta americana* Linn.	Lab. of Insect Path., U.C.	Acc.593
55–1–1	*Cylas formicarius elegantulus* (Sum.)	Lab. of Insect Path., U.C.	Acc.615
56–1–1	*Oryctes rhinoceros* Linn.	Lab. of Insect Path., U.C.	Acc.637
61–1–1	*Gnorimoschema gallae-solidaginis* (Riley)	Lab. of Insect Path., U.C.	Acc.731
63–1–1	Eggs of *Dacus dorsalis* Hendel	Lab. of Insect Path., U.C.	Acc.766
68–1–1	*Agrotis ypsilon* (Rott.)	S. B. Chattopadhyay	A-3
73–1–1	*Heliothis zea* (Boddie)	Lab. of Insect Path., U.C.	Acc.1061
73–2–1	*Heliothis zea* (Boddie)	Lab. of Insect Path., U.C.	
76–1–1	*Tenebrio molitor* Linn.	Lab. of Insect Path., U.C.	Acc.962
78–1–1	*Dendroctonus monticolae* Hopk.	Lab. of Insect Path., U.C.	Acc.1223
79–1–1	*Pericyma cruegeri* Butler	Lab. of Insect Path., U.C.	Acc.1274

make much difference if they fed on this mixture for only 40 hours (after which they were fed bacteria-free food) or continuously for the duration of the experiment (15 days)—the total mortality remained about the same. An exception to this, however, occurred in the controls of another type of experiment in which strain 0–8–13 infected 16 out of 25 larvae (64 per cent) which ingested the bacterium along with their food.

In an experiment with *Peridroma* larvae it was found that, using 10 insects for each test, microfeeding *S. marcescens* (strain 0–8–13) resulted in a mortality of 30 per cent, feeding foliage dipped in the bacterium killed 50 per cent, and inoculating by direct puncture caused a 90 per cent mortality. Parallel figures for similar tests in *Sabulodes* larvae were percentages of 20,

50, and 70. Just why the more direct microfeeding appeared to be less effective in promoting infection than the feeding of foliage that had been dipped in the bacterium is difficult to say, but it is an interesting phenomenon that we have noticed on other occasions, although frequently the situation is reversed. Combining the data obtained in tests using strains 0–8–2 through 0–8–11 (as given in Table 1), it was observed that when microfed to *Peridroma,* deaths occurred with only 3 of the 10 strains tested with an average percentage mortality of 27; when fed with foliage, deaths occurred with 6 strains with an average percentage mortality of 18; but when microinjected, 9 of the 10 strains were pathogenic with an average mortality of 83 per cent. In a later experiment in which strain 0–8–13 was fed on foliage to 33 *Peridroma* larvae, 12 (36 per cent) died of *S. marcescens,* and of 20 *Sabulodes* larvae similarly fed, 11 (55 per cent) died.

Similar comparative tests were completed using larvae of other insects. In the case of *Junonia,* 16 strains (0–8–2 through 0–9–4) were tested. By microfeeding, 6 of these strains were capable of causing death with an average mortality of 23 per cent, by ingestion 6 strains caused a mortality of 10 per cent, and when microinjected all 16 strains were pathogenic with an average mortality of 91 per cent. Using 5 strains (0–8–2 through 0–8–6), larvae of *Galleria* were susceptible to 3 strains (12 per cent mortality) when microfed, 2 strains (6 per cent mortality) when ingested, and all 5 strains (80 per cent mortality) when microinjected. When the same 5 strains were tested in *Colias* the results showed this insect to be susceptible to 4 strains (25 per cent mortality) when microfed, 5 strains (32 per cent mortality) when ingested, and 5 strains (96 per cent mortality) when microinjected. In other experiments with *Colias,* it has been noticed that at times the mortality after an infectious feeding (foliage dipped in a bacterial suspension) may be as high as 90 per cent.

In a preliminary experiment, with 20 *Bombyx* larvae in each test, it was found that *S. marcescens* strain 0–8–13 was capable of causing a mortality of 30 per cent when microfed, 5 per cent when ingested directly from the end of a dissecting needle, 85 per cent when microinjected, 100 per cent when punctured with needle bearing bacteria directly from solid media rather than an aqueous suspension of the bacteria, and no mortality occurred in those silkworms which had been fed the bacteria on mulberry leaves. (In a subsequent experiment in which strain 0–8–13 was fed to *Bombyx* larvae along with mulberry leaves, a mortality of 20 per cent resulted.) On another occasion 31 strains (0–8–2 through 76–1–1) were tested in *Bombyx.* The silkworms were susceptible to 15 strains (29 per cent mortality when microfed, 12 strains (13 per cent mortality) when ingested, and all 31 strains (87 per cent mortality) when microinjected. In the case of the microinjection tests, 20 of the 31 strains caused a mortality of 100 per cent; the lowest per cent mortality was 20 per cent, for strain 0–8–6.

The only insect not a Lepidoptera used in the present investigation was the coleopterous *Tenebrio molitor* Linnaeus. Susceptibility tests in larvae of this insect, the yellow mealworm, using strains 0–8–2 through 0–8–6, and strain 76–1–1, were run in such a manner as to compare the mortalities following microfeeding (0.006 ml), microinjection (0.003 ml), and contaminated food-

feeding. In each test, 10 larvae were used for each route of inoculation. The results, as shown in Table 2, were fairly uniform as between strains. As might be expected, the percentage of deaths from intrahemocoelic inoculation was very high (99 per cent) and that from food-feeding very low (6 per cent), but that (75 per cent) occurring in the microfed insects, while not as high as that occurring by direct inoculation into the body cavity, was nevertheless considerably higher than that occurring in the case of the lepidopterous larvae. It should be remembered, however, that this generalization is

Table 2. Average precentage mortalities (roughly estimated) caused by various strains of *Serratia marcescens* in larvae of different lepidopterous insects and the beetle *Tenebrio*, according to manner of administration. Data taken from numerous different experiments, and subject to qualifications indicated in text.

Insect	Manner of administration		
	Microfed	Food-fed	Intrahemocoelic inoculation
Colias	25	52	96
Junonia	23	38	91
Bombyx	28	13	91
Peridroma	28	35	87
Pseudaletia	..	8	..
Sabulodes	20	58	75
Galleria	12	20	82
Tenebrio	75	6	99

based upon fewer strains and fewer data than in the case of most of the tests with Lepidoptera. Also, it is possible that some degree of injury to the insect's gut wall may occur during the microfeeding operation thus enhancing the chance of direct infection through the alimentary tract. The fact that the results of these tests indicate a higher susceptibility of the mealworm to *Serratia marcescens* than those obtained by Masera (1934*b*) is difficult to explain except that we were working with entirely different strains of the bacterium.

It is rarely safe to make generalizations from miscellaneous data collected at different times, under different conditions, using different strains of *S. marcescens* and different rearings of insects. Nevertheless, it might at least be interesting to so gather all our data, not only from the experiments just recounted but from some of our other experiments as well, and to average the per cent mortality according to the manner in which the bacterium was administered. These figures, which should be accepted with appropriate qualifications, are presented in Table 2. It should be emphasized that throughout this study it was observed that not only was there considerable variation in the pathogenicity of *S. marcescens* according to the particular strain of bacterium involved, but that any one strain gave variable results when tested at different times in the same insect species. Even when all possible attempts were made to standardize the quality and dosage of the bacterial strain used, the insect, and the environmental conditions, there still appeared marked

inconsistencies in the amount of mortality that resulted in individual experiments—at least this was the case with most of the insects included in this investigation.

Enhancement of Virulence

Although it was not intended that this investigation should include a comprehensive study of the possibility of increasing the virulence of given strains of *S. marcescens* for insects, we did conduct some experiments along this line that are worth reporting.

Experiment No. 1. *S. marcescens* strain 0–8–4 which, in the general susceptibility tests, killed 80 per cent of the *Galleria* larvae when microinjected, but none when ingested or microfed, was selected for this experiment. Bacteria grown for 24 hours on nutrient agar were microfed to 10 *Galleria* larvae. One of these insects died with a *marcescens* septicemia; the blood from this insect was used as an inoculum and microfed to 10 fresh larvae. Similar transfers were carried out until upon the tenth transfer none of the 10 test larvae became infected. The number of deaths occurring with each successive transfer to 10 fresh insects were as follows: 1, 8, 4, 2, 9, 4, 2, 8, 4, and 0. Thus it is clear that, in this experiment, there was no steady increase in the virulence of the strain concerned as indicated by the mortality occurring with each successive transfer. Indeed the vacillation in numbers killed was great. Once the mortality reached 90 per cent (on the fifth transfer) it did not remain at this level, but continued to rise and fall upon successive host to host transfers. Nevertheless, the infectious blood appeared to be more reliably pathogenic than bacteria grown on nutrient agar as evidenced by the relative ease in making the two types of transfers in this and other experiments.

With the third transfer some of the infective *Galleria* blood was also microfed to 5 *Tenebrio* larvae. Three of these died. However, upon transferring blood from one of these dead insects to 10 fresh *Tenebrio* larvae, none became infected. Transferring blood from the same insect to 2 *Galleria* larvae resulted in 2 deaths, but upon the next transfer to *Galleria* larvae none became infected.

An attempt to repeat this experiment starting with the blood from an infected *Galleria* larva failed after two transfers when none of the 10 recipient larvae showed any signs of infection. A third attempt was then made using strain 0–8–7. The number of deaths occurring with each successive transfer were 2, 10, 4, 3, 5, 0. No infections occurred following the sixth transfer. Thus, not only was there no apparent increase in the virulence of the bacterial strains used, but the infection could not be maintained by serial transfer (microfeeding) of blood from freshly dead larvae.

Experiment No. 2. Preliminary tests had indicated that passage of *S. marcescens* (0–8–4) through *Tenebrio* larvae somewhat enhanced the virulence of the bacterium for *Galleria* upon subsequent direct transfer. Further experiments gave some credence to this indication, but not in a convincing manner nor was the increase, if any, maintained upon subsequent transfer through *Galleria* larvae. Thus, in one instance, infectious *Tenebrio* blood was microfed to 5 *Galleria* larvae all of which died of infection; upon the next transfer (to 10 *Galleria* larvae) all of these also died; but upon two further

transfers only 5 of 10 died. In another similar series, the infectious *Tenebrio* blood mortally infected 3 out of 5 *Galleria* larvae, upon the next transfer 7 out of 10 larvae died, then 4 out of 10.

Once again strain 0–8–4 was injected into 10 *Tenebrio* larvae, all of them dying within 30 hours. The blood from one of these larvae, when microfed to 10 *Galleria* larvae, killed only one, upon the next transfer again only one died, then 3, then 6, then 3, and upon the sixth transfer none of the *Galleria* larvae became infected.

In another test, *Tenebrio* larvae were inoculated directly with strain 0–8–4. The blood from this insect, which died in 24 hours, was inoculated directly into the body cavities of 5 *Tenebrio* larvae, all of which were dead after 24 hours. From one of these insects blood was transferred by intrahemocoelic inoculation to 10 *Tenebrio* larvae. All 10 died within 24 hours. However, when the blood for 1 of these larvae was microfed to *Galleria* larvae, only 1 out of 10 died, although 5 out of 10 *Tenebrio* died upon this microfed transfer, and 2 out of 10 died upon the subsequent transfer.

In the meantime, serial transfers through *Tenebrio* alone were giving results as erratic and as indefinite as those described in Experiment No. 1 with *Galleria*. Serial passages (microfeeding) were rarely successful for more than 6 or 7 transfers, and frequently only 1 or 2 transfers were successful.

Incidentally, in a single experiment it was found that strain 0–8–4, when grown for 48 hours on Bombyx-blood-serum agar showed no increase in virulence for *Bombyx* when microfed to the larvae. Also, in a single experiment in which *Tenebrio* larvae were exposed to ultraviolet light (GE-15 watt Germicidal Lamp; 2537Å) for 10 minutes at a distance of 6 inches, only slight if any increase in susceptibility to *S. marcescens* was observed.

Miscellaneous Tests

During the course of this study, a number of experiments were conducted in a limited and preliminary fashion only. Most of them bear repeating, and the results should be considered provisionary. Nevertheless, the results do appear to be indicative, and at this point we have no reason to question their basic validity.

Use of Abrasives. In 1956 (see Steinhaus, 1958*b*) we reported the results of an experiment in which triturated glass was fed continuously to silkworm larvae together with a strain (0–8–13) of *S. marcescens*. The resulting mortality amounted to approximately 70 per cent as compared with 20 per cent when *S. marcescens* alone was fed, and 0 per cent when triturated glass alone was fed. Weiser and Lysenko (1956) using the bacterium *Pseudomonas noctuarum* (White) (now believed by Lysenko (1958) to be a strain of *S. marcescens*), reported similar results in silkworms. One of the ultimate objectives of such experiments would be to determine the feasibility of increasing the effectiveness of a pathogen by enabling it to penetrate to the body cavity of the insect after the lining of the alimentary tract has been scarified or lacerated with abrasives. Since 1956, we have conducted limited experiments using other abrasives, as well as crushed glass. The results of some of these experiments are reported here.

Experiments similar to those previously reported with the silkworm were

performed using *Galleria* larvae. The triturated glass was prepared by finely pulverizing glass tubing with a pestle and mortar. In one experiment *S. marcescens* (strain 0–8–7) plus glass killed an average of 5 out of 10 larvae in each of five transfers, while no successive transfers after microfeeding the original 10 larvae were accomplished with *S. marcescens* alone. In another test, using *S. marcescens* strain 0–8–4, the bacterium plus glass killed 2, 3, 3, and 6 out of 10 in each of four successive transfers; only one successful transfer (2 deaths) was effected using bacteria alone. In a third experiment, 14 out of 25 larvae (56 per cent) fed *S. marcescens* (strain 0–8–13) plus glass along with the insect's food, and 16 (64 per cent) fed only *S. marcescens*, died of infection. Transfers to additional series of larvae were not made in this instance. While these data are insufficient to form a definite conclusion, they do appear to indicate that triturated glass tends to enhance the likelihood of infection by *S. marcescens* in *Galleria* larvae when the abrasive and bacteria are microfed, but not when they are fed along with the insect's food. Unfortunately, it was necessary to use different strains in these tests, so some of the difference may be caused thereby, although the latter conclusion would not be warranted on the basis of our knowledge of the comparative pathogenicity of the strains concerned.

In a similar series of experiments using *Junonia, Colias, Peridroma,* and *Sabulodes*, triturated glass alone (fed either along with the insects' food or by microfeeder) induced no appreciably increased amount of disease in larvae of these insects. When *S. marcescens* (strains 0–8–9, 0–8–13, and 55–1–1) was added to the glass, no significant increase in infections over the controls was noted. Withholding food from the insects for 24 to 48 hours prior to the tests did not appear to make any difference.

Following our experiments with triturated glass, we explored the possibility of using other types of abrasives. It was decided to perform a few preliminary experiments using the well-known abrasive silicon carbide.[4]

At the outset, difficulties were experienced in administering the sodium carbide preparations to the test insects by means of the usual hypodermic microinjection syringes which would bind or stick, preventing free movement of the plunger. Eventually a syringe with a specially constructed plunger was devised, which avoided the mechanical difficulties involved in using ordinary glass plungers. Until this device was available, however, it was decided to permit most of the abrasive particles to settle (for about 20 minutes) to the bottom of the suspension, and to use primarily the supernatant, in the hope that the number of particles remaining would be small enough and not so great in number as to prevent binding the syringe and yet exert some abrasive action in the insect gut.

Using larvae of *Tenebrio molitor,* suspensions of *S. marcescens* (strain 0–8–7) alone, and suspensions of *S. marcescens* plus the supernatant from small amounts of silicon carbide, Grit No. 600, were microfed in parallel series of 10 larvae for each transfer. (This experiment was not done on an accurate quantitative basis. Roughly the infective blood from 1 insect was diluted with

[4] This was kindly furnished us by the Electro Minerals Division of the Carborundum Company, Niagara Falls, New York. In most of our experiments the Carborundum Company's Grit No. 600 (average size: 16 microns) and Grit No. 1000 (7 microns) were used.

2 ml of water; to this was added approximately 0.18 gram of abrasive.) The series of *Tenebrio* larvae that received *S. marcescens* alone showed a mortality of 6 out of 10 upon the original microfeeding, 7 out of 10 upon the next (blood) transfer, and 0 out of 10 upon the following transfer. On the other hand, the series of larvae that received *S. marcescens* plus the silicon carbide showed moderate to high mortality throughout 17 successive transfers, after which the experiment was closed. The number of larvae (out of 10) dying upon each transfer were as follows: 7, 3, 6, 7, 7, 8, 9, 5, 10, 7, 2, 9, 8, 10, 10, 9, and 7.

In a similar experiment using strain 0–8–4 and Grit No. 1000, rather similar results were obtained. The *Tenebrio* larvae that received *S. marcescens* but no abrasive showed a mortality of 6 out of 10 upon the original microfeeding, 2 out of 10 upon the first (blood) transfer, and upon the next six transfers 1, 6, 1, 1, 1, and 7 out of 10. Upon the eighth transfer no mortality resulted. In the series of larvae that received both *S. marcescens* and the silicon carbide supernatant, the mortality figures for the original microfeeding and for each transfer were as follows: 2, 3, 4, 3, 10, 10, 10, 4, 9, 1, 4, 7, 10, 10, 10, 3, 8, 10, 10, 9, 8, 7, 6, 4,—a total of 23 transfers before the experiment was voluntarily stopped.

In a third experiment, in which original microfeedings were compared but serial transfers not made, it was found that *S. marcescens* (0–8–4) alone killed 11 out of 20 *Tenebrio* larvae, the bacterium plus the silicon carbide supernatant killed 17 out of 20, and the supernatant alone killed none of 20 larvae. Somewhat later, this experiment was repeated, with the bacterium alone killing 12 out of 40 larvae, the bacterium plus the silicon carbide supernatant killing 24 out of 40, and the supernatant alone killing none of 40 larvae.

These experimental results, which need confirmation, are difficult to interpret. It appears that the addition of the supernatant from an aqueous suspension of silicon carbide (but still containing numerous particles of the latter) to an inoculum of *S. marcescens* increases the mortality resulting from infection with this organism over that occurring when the bacterium alone or the supernatant alone is used. This could be a result of the invasive action of *Serratia* following the abrasive action of the particles of silicon carbide remaining in the supernatant, or it could represent the effect of some chemical or soluble substance eluted from the carbide which enabled the bacterium to invade the insect's body cavity. Which, if either, of these factors is involved has not been determined.

Use of Chemicals. It is not our purpose, in the present paper, to include a detailed account of our experiments on the use of chemicals to increase the likelihood of *S. marcescens* infecting a given insect. However, some results, of a preliminary nature, are worth reporting at this time.

In an initial experiment, *S. marcescens* (strain 0–8–4) was suspended, as previously in water, in a 0.06 N solution of acetic acid and microfed in 0.006 ml amount to each of 10 *Galleria* larvae. Within 3 days, all 10 larvae had died with a *marcescens* septicemia. Ten control larvae microfed with acetic acid only remained healthy; the mortality with *S. marcescens* alone was 1 out of 10. When the concentration of 0.03 N acetic acid (and the bacterium) was

used, 5 out of 10 larvae died. On the basis of this single experiment it would appear that in the case of *Galleria*, acetic acid somehow enabled *S. marcescens* to be a more effective pathogen. Similar results were not obtained in two such experiments using *Tenebrio* larvae, and in one experiment using *Bombyx, Colias, Peridroma,* and *Pseudaletia* larvae.

In a second experiment, essentially similar results were obtained: *S. marcescens* (9–8–4) microfed alone to *Galleria* killed 3 larvae within 7 days, appear that in the case of *Galleria,* acetic acid somehow enabled *S. marcescens* killed 3 with septicemia, but when the bacterium was microfed with 0.06 N acetic acid, 9 out of 10 larvae died of infection within 24 hours after inoculation. If the bacteria suspended in 0.06 N acetic acid is added to the food of the larvae, no increase in mortality results. In fact, in the experiment in which this was tested, none of the 10 test insects became infected.

Several months after the two experiments just cited were performed, two additional but similar experiments failed to confirm these results, there being no essential difference in the number of deaths in the group receiving *Serratia* in 0.06 N acetic acid and in that receiving the bacterium alone. Furthermore, similar experiments using butyric acid, lactic acid, and proprionic acid showed only a slight, and probably not significant, increase in mortality when these chemicals were combined with *Serratia* over that occurring when the bacterium was used alone.

A preliminary experiment was conducted in which 10 per cent soluble starch was microfed along with *Serratia.* This combination gave only a slightly higher (if any) percentage mortality than did the bacterium alone. A 50 per cent glycerin emulsion also failed to cause any substantial increase in mortality from *Serratia.*

Use of Heat and Moisture. As previously reported (Steinhaus, 1956) we have observed the body cavity of some insects to be invaded with bacteria from the gut when the larvae are held at higher than normal temperatures (e.g., 40°C). It has also been noted that high temperatures may also enhance the susceptibility of insects to bacteria administered intentionally.

In the case of *S. marcescens,* the role of heat in enabling the bacterium to infect insects is not clear. In a number of different experiments using larvae of *Colias, Sabulodes, Junonia, Estigmene,* and *Galleria,* only exceptionally does an increased incidence of *S. marcescens* infection occur in larvae held at 40°C when the bacterium is administered along with the insects' food. Indeed, at times there is less infection at 40° than at 37° or 33°C, or at room temperature (21 to 25°C). The number of deaths in the test lots rarely exceeded those in the controls held at the same temperature; the deaths in the latter may have been the result of septicemias caused by invading gut bacteria, those in the former by a combination of gut bacteria and *S. marcescens.*

These results might be explainable by the fact that most strains of *S. marcescens* do not grow at temperatures above 37°C. The optimum range is between 25° and 30°C.

An experiment in which *Tenebrio* larvae were subjected to 2°C for 3 days, then fed *S. marcescens* (0–8–4) did not give any indication that the low-temperature exposure increased the insect's susceptibility to the bacterium.

In a limited experiment testing the effect of atmospheric moisture on *S.*

marcescens (0–8–13) infection in *Galleria*, a slight increase in mortality was noticed at relative humidities of almost 100 per cent over that occurring at relative humidities of about 55 per cent. *Tenebrio* larvae held at a temperature of 33°C and a relative humidity of 82 per cent for 3 days prior to being fed *S. marcescens* showed no appreciable increase in mortality over that occurring in larvae similarly held at the usual relative humidity of the room, i.e., 55 per cent. Similar results were obtained when the relative humidity was reduced to 20 per cent, at a temperature of 33°C.

Microfeeding *Peridroma* larvae excessive quantities of water, every day for 5 days, alone and with *S. marcescens* did not appear to affect significantly the incidence of mortality. This experiment was run because of what appeared to be some indication that excessive amounts of water in the food of some insects caused them to develop diarrhea presumably promoted by the abnormal development of bacteria in the gut.

Transmisison by Contact. During the course of experiments on the role of crowding as a possible stress factor in insect disease (Steinhaus, 1958*a*), an effort was made to determine whether or not a bacterium such as *S. marcescens* may be transmitted from infected to healthy larvae by contact between diseased and healthy insects. Experiments were set up in duplicate using *Colias* and *Peridroma* larvae, 10 insects to a carton (13.5 cubic inches). To each of the first two cartons, 1 infected (by microfeeding strain 0–8–13) larva was added; to each of the second two cartons, 2 infected larvae were added, to each of the third two cartons, 3 infected larvae were added, and to each of the fourth two cartons, 4 infected larvae were added. The added infected larvae were tagged with eosin to differentiate them from the others.

In the case of *Peridroma* the incidence of *S. marcescens* infection in the originally uninfected larvae increased only slightly, and this mostly in the carton that had received the 4 infected specimens, 5 additional larvae out of a total of 20 (i.e., 25 per cent) succumbing to infection after about 10 days.

On the other hand, in the case of *Colias* transmission of the bacteria by contact was more evident. Discounting the deaths of the tagged larvae, approximately 43 per cent of the insects acquired sufficient *S. marcescens* by contact (or through frass contamination) to cause infection and death. In the case of the cartons receiving 4 infected caterpillars each, 90 per cent succumbed to *S. marcescens* infection within 8 days. In another experiment in which each of 25 infected *Colias* larvae were placed, one to a carton, with an uninfected larva, transmission occurred in at least 76 per cent of the cases. In both *Colias* experiments it appeared that the longer a tagged (infected) larva lived the more likely it was that the larvae it was with would contract the infection. The exact nature of the transmission was not determined but it is assumed that the bacteria were disseminated largely through the insect's excretions which contaminated the food (foliage) and carton.

Double Infection. Incidental to testing the susceptibility of lepidopterous larvae to *S. marcescens*, an attempt was made to determine if the simultaneous infection of *Galleria* larvae with *S. marcescens* and *Bacillus thuringiensis* Berliner causes a greater mortality in this insect than when either bacterium is administered alone. The bacteria were fed to the larvae by incorporating the microorganisms in the insect's food. A viable spore prepa-

ration of *B. thuringiensis* and a 36-hour culture of *S. marcescens* (0–8–13) were employed in these experiments. Two experiments were run. Of the 100 larvae used in each experiment, (a) 25 were fed *B. thuringiensis* only, (b) 25 were fed *S. marcescens* only, (c) 25 were fed a combination of the two bacteria, and (d) 25 were held as uninfected controls.

In the first experiment, over a 16-day period there were 11 deaths caused by *B. thuringiensis* in group (a), 5 caused by *S. marcescens* in group (b), in group (c) all 15 that died had *S. marcescens* in their hemolymph. No deaths occurred in group (d), the controls. In the second experiment, again over a 16-day period, 19 of group (a) died of infection with *B. thuringiensis*, 3 of group (b) died of *S. marcescens* infection, and in group (c), 6 of the dead larvae showed both *B. thuringiensis* and *S. marcescens* in the body cavity, 15 showed only *S. marcescens,* and 3 showed only *B. thuringiensis.* None of the 25 controls died.

From these results it would appear that in *Galleria* while *S. marcescens* may inhibit the development of *B. thuringiensis* when fed to the same individual (possibly because of the antibiotic action of the former), the latter nevertheless enables *S. marcescens* to develop more freely. Since this phenomenon occurs even when the vegetative rods of the bacillus are not in evidence, it may be that it is the toxic crystalline inclusion formed at the time of sporulation and administered with the spores that is responsible for increasing the insect's susceptibility to *S. marcescens* or enabling *S. marcescens* to be a more effective secondary invader.

DISCUSSION

Serratia marcescens Bizio, as far as its relation to insects is concerned, appears to be one of those bacteria that belong to the arbitrary group we have designated (Steinhaus, 1958c) as nonsporeforming facultative pathogens. It is not a true entomogenous organism as far as its natural habitat is concerned. It is generally recognized as being saprophytic on decaying plant and animal matter and occurring widely in water, soil, and air; it is commonly found on bread, meat, milk, potatoes, and other food stuffs. The data presented in this paper do not explain why *S. marcescens* is a facultative or "sometimes" pathogen for insects, nor do they reveal how the bacterium may reliably be made to act as an obligate pathogen. Nevertheless, we have confirmed the fact that, under certain conditions, *S. marcescens* can and does cause disease in insects.

Our studies on *S. marcescens* as an insect pathogen have not proceeded far enough to include in this paper a report of our findings pertaining to the possible mechanisms involved. As in most microbial diseases, we are concerned with three general factors or natural entities: the bacterium, the insect host, and the external and internal environment. The variable attributes of each of these are so intertwined that it is as difficult as it is unrealistic to attempt to study them separately. When we seek to determine the mechanisms involved in an infection of a particular insect by a particular bacterium, we can at best examine certain likely dominant processes, hoping that in so doing we shall discover clues to specific factors or mechanisms. We must be careful that when we speak of the susceptibility of a given

insect to a given bacterium we make clear whether we are concerned with the attributes of the host only (with its higher or lower resistance to invasion), or also with the attributes of the bacterium with its varying capacity to invade and cause disease in the host, or both. Both of these, of course, are influenced by a changing environment (see Steinhaus, 1954). When we can at will change any of these attributes it is possible that we thereby can change the balance between them, increase or decrease the virulence of the pathogen, increase or decrease the susceptibility of the host, and alter the environment. One way of accomplishing these changes is through the use of stressors and/or incitants.

As is shown by the data presented in this paper, as well as those given by other workers, the susceptibility of insects to infection by *S. marcescens* may be thought of as being of at least two different types: (1) there is a difference in susceptibility as among species, (2) and there is an apparent difference in susceptibility as among individuals. The relative inconstancy with which a particular strain of *S. marcescens* is pathogenic for a particular species of insect is difficult to explain even theoretically. The same is true as it pertains to individual susceptibilities. The latter may not be a true difference in that the particular host individual is different or changed (i.e., less susceptible than another). More likely it reflects a difference or change in the way the three factors (pathogen, host, environment) with their variable attributes coalesce or intertwine to bring about a particular consequence. The fact remains that in numerous instances a greater or lesser number of individuals of a given insect species will succumb to adequate doses of orally administered inocula, while the remainder of a sufficiently large sample will survive. The question naturally arises: "Why?" When, for example, in a given experiment we find that out of 20 *Bombyx* larvae microfed the bacterium, 6 die of septicemia, we wonder what happened in these 6 that did not happen in the remaining 14, or vice versa.

Naturally, the conditions prevailing in the alimentary tract are worthy of primary consideration. The hydrogen ion concentration of the gut contents is conceivably important in determining the general susceptibility of an insect species to the bacterium; but unless this factor varies considerably among individuals (as it is sometimes known to do), it does not help a great deal in explaining individual susceptibility. In addition, the nature of the food ingested, moisture content of the gut, the digestive fluids and secretions, the presence of bactericidal substances such as those reported by Masera (1954) and referred to earlier in this paper, the frequency of gut elimination, the nature of the gut wall, and other factors all need consideration in this matter.

Some of the pathogenicity of *S. marcescens* for certain insects might simply be a reflection of the ability of the bacterium to reproduce in large numbers in the gut and body cavity of the insect. Its ability to do this may depend largely upon the conditions it finds prevailing in the particular insect concerned, as well as on dosage. In some cases the bacterium multiplies to extraordinarily great numbers in the gut, and this tremendous growth could well exert pressures of various sorts on the bacterium-host balance.

Conditions in the alimentary tract of insects can, of course, be altered. In a preliminary way, we have sought to do this through the use of abrasives such as silicon carbide and triturated glass. The latter substance appeared to enhance the likelihood of infection by *S. marcescens* in some insects (*Bombyx*, *Galleria*), but not in others. At this point we can only assume that the action of the glass in an insect such as the silkworm is such as to scarify the gut membranes thus permitting ready entrance of the bacteria into the body cavity. If this were the case, however, one wonders why some of the bacteria normally in the gut did not, in the controls, similarly enter the body cavity and produce a septicemia. The answer might be that the intestinal flora of the silkworms we used was very sparse, and that the scarification caused by the glass was enough to permit the penetration of a slightly aggressive bacterium like *S. marcescens* but not those that happened to be in the gut. In the case of those insects (*Junonia*, *Colias*, *Peridroma*, and *Sabulodes*) that exhibited no unusual increase in infection, it is possible that the triturated glass used was not as effective in scarifying the gut tissues as in *Bombyx*.

The fact that *S. marcescens* suspended in dilute acetic acid sometimes increased the percentage mortality when fed to *Galleria* is also difficult to explain at this time. We assume that the acid affects the host or conditions in the host more than it affects the bacterium. It may simply be a matter of altering the pH of the gut, or possibly it may inhibit a bactericidal principle in this organ.

As far as the bacterium itself is concerned, the evidence seems clear that there is some variation in the virulence of the 33 different strains of *S. marcescens* used in this investigation. Some strains, such as 0-8-7, 0-9-1, and 0-11-3, appeared to be relatively more virulent than other strains, such as 0-8-3 and 0-8-9, for most of the insects included in our study. However, this difference does not appear to be consistent or great. If the differences that have been observed are a reflection of an inherent capacity of the bacterium to invade, this factor is either not stable or, more probably, is easily influenced or affected by other factors. So far we have obtained no evidence of a true exotoxin being produced, but our tests for this have been cursory and inadequate. The possibility should also be recognized that strain differences and variations (both with respect to the bacterium and the insect host, *e.g.*, in the case of the silkworm), may account for some of the differences in the results obtained by different workers in susceptibility tests run by them.

In any speculation as to the role of the infectious agent in a disease, the matter of dosage must, of course, always be considered. In this regard, *S. marcescens* is no different from many other bacterial facultative pathogens of insects: the greater the dosage the more likely it is that infection will result. Not only do greater dosages increase the amount of growth that occurs in the gut of an insect, but they also enable the bacterium to overcome inhibitory influences in the gut and to produce greater amounts of toxic substances and enzymes. As far as the variations in virulence referred to here are concerned, however, the matter of dosage is not believed to play a role

since this factor was standardized as indicated in the section on methods. Similarly, in experimental work, the manner of administering the inoculum is important and significant. It is our belief that direct inoculation of a bacterium such as *S. marcescens* directly into the body cavity of an insect tells very little relative to the microorganism's natural capacity to invade the animal. Occasionally one does encounter an organism that is unable to develop in the body cavity and this is a good indication that it has little innate pathogenicity for the insect. On the other hand, most gram-negative rods grow readily (and destructively) in the hemolymph of insects and, when they do, this is about all that is revealed since it is very little or no indication that the bacterium is pathogenic for the insect via the normal portals of entry. Certainly, in our experiments on transmission of *S. marcescens* by contact, the rate of infection more closely approximates that we had obtained by methods of oral administration than by puncture and intrahemocoelic inoculation methods.

One possibility that does need to be seriously considered involves the ability of *S. marcescens* to produce the enzyme lecithinase. In the case of certain strains of *Bacillus cereus* Fr. and Fr., Heimpel (1955*b*) has shown that a significant correlation exists between the pathogenicity of different strains of *B. cereus* for the larch sawfly, *Pristiphora erichsonii* (Htg.), and their abilities to produce lecithinase. Monsour and Colmer (1952) clearly demonstrated that 7 strains of *S. marcescens* they studied exhibited lecithinase "C" activity in an egg yolk suspension. Arnaudi and Novati (1957) studied a strain of *S. marcescens* that had licithinase A, B, and C activities. Since, on the basis of Heimpel's work, it might be reasonable to expect that strains producing the greatest amount of lecithinase should be the most virulent, and vice versa, it is possible that some of the variation we have observed in virulence of the different strains of *S. marcescens* could be caused thereby. Of course, the hydrogen ion concentration of the midgut of the insect concerned would have to be within the pH range of lecithinase activity. We have not had the opportunity to test the lecithinase productivity of our *S. marcescens* strains, but obviously, it should be done together with the pH of the gut contents of the insects concerned.

Admittedly, our attempts to enhance the virulence of certain strains of *S. marcescens* by passing them through succeeding host individuals has been somewhat disappointing. When the virulence appeared to have been enhanced by this procedure, it did not maintain itself at this high level. At best the increased virulence even for the host used for passage was largely transitory. In light of the claims of Paillot, d'Herelle, Metalnikov, and others with other bacteria, our discouraging results are somewhat puzzling. Perhaps we need to run more tests with more strains, although the number of tests run and the number of strains used appeared to us to be reasonably adequate.

Among the environmental factors to be considered are heat and moisture, measured as temperature and relative humidity. In this area our experiments are admittedly relatively cursory and incomplete. As far as we have gone, it does not appear that abnormally high or abnormally low temperatures and humidities appreciably increase the mortality from *S. marcescens* infection. Nevertheless, it is possible that adverse temperatures and humidities

might have a debilitating effect on insects and possibly make them more susceptible to infection. The role of environmental stress in disease caused by this bacterium warrants a broad and complete study.

SUMMARY

1. From a review of the literature, it appears that as yet the reputed role of the red-pigmented bacterium *Serratia marcescens* Bizio as a pathogen of insects is based largely on the occurrence of outbreaks among laboratory or insectary-reared insects and on experimental infections rather than on any marked degree of spontaneous infection among insects in nature.

2. The present paper is a report on miscellaneous studies designed to initiate an inquiry into what extent the occurrence of *Serratia* infections is related to factors of stress that may be present under certain laboratory conditions but do not necessarily prevail in nature. If this matter can be clarified, we can then ask whether these stressors could be applied in the field so as to make the bacterium a more effective pathogen, and possibly a potential control agent.

3. Using various strains of *S. marcescens* from an accumulation of 33—mostly entomogenous in origin—general susceptibility tests and other experiments were run on 9 species of insects belonging to the genera *Colias, Junonia, Bombyx, Estigmene, Peridroma, Pseudaletia, Sabulodes, Galleria,* and *Tenebrio.*

4. In general, we have been able to confirm the commonly held concept that while *S. marcescens* is highly pathogenic for many insects upon direct inoculation into the body cavity, it is considerably less so, and in some instances only slightly pathogenic, when fed to them. There may, however, be considerable variation in the pathogenicity of *S. marcescens* for certain insects according to the strain of bacterium involved. Moreover, any particular strain may not be consistent in the percentage mortality resulting at different times in a particular insect species. Nevertheless, data as to the general susceptibility of the insects named to different strains of *S. marcescens* are presented.

5. Attempts to increase the virulence of certain strains of *S. marcescens* for the test insects by repeated passage through them were not consistently successful. While some apparent enhancement in virulence could be attained after a few passages, this could not be continuously maintained.

6. The use of some abrasives, such as triturated glass, appeared to increase significantly the likelihood of infection by *S. marcescens* in some insects (in *Bombyx* when microfed and food-fed, and in *Galleria* when microfed but not so clearly when food-fed). No appreciably increased amount of disease was induced in this manner in *Junonia, Colias, Peridroma,* or *Sabulodes* in limited tests.

7. When *S. marcescens* is suspended in a solution of dilute acetic acid and microfed to *Galleria* larvae, the resulting mortality from infection may at times show some increase over that resulting from the administration of *S. marcescens* alone. The data obtained need confirmation. Starch and glycerin caused no substantial increase in mortality from *Serratia.*

8. Abnormally high or abnormally low temperatures and humidities do not appear to increase very appreciably the mortality from *S. marcescens* infection when the bacterium is administered to the test insects during or following these treatments.

9. In the case of some insects (e.g., *Colias*), it was shown that transmission of the bacterium by contact (between diseased and healthy insects) may occur when infected larvae are placed among groups of healthy larvae. Transmission also occurs frequently (76 per cent) when a single healthy larva is placed in the same container with a single infected larva.

10. In *Galleria,* while *S. marcescens* may inhibit the development of *Bacillus thuringiensis* when fed to the same individual, the latter (or its crystalline toxin) enables *S. marcescens* to develop more freely or to act more easily as a secondary invader.

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